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(51) International Patent Classification ⁶: C12Q 1/68, 1/00, C12P 21/06, C12N 15/00, 1/20, G01N 33/566, C07K 3/00	A1	(11) International Publication Number: WO 95/06754
		(43) International Publication Date: 9 March 1995 (09.03.95)
(21) International Application Number: PCT/US94/09898 (22) International Filing Date: 1 September 1994 (01.09.94) (30) Priority Data: 08/115,161 1 September 1993 (01.09.93) US (71) Applicant: THE REGENTS OF THE UNIVERSITY OF CALIFORNIA [US/US]; 22nd floor, 300 Lakeside Drive, Oakland, CA 94612 (US). (72) Inventors: KUSHNER, Peter; 1362 - 6th Avenue, San Francisco, CA 94122 (US). WEBB, Paul; 1300 Irving Street #21, San Francisco, CA 94122 (US). (74) Agents: BASTIAN, Kevin, L. et al.; Townsend and Townsend Hourie and Crew, Steuart St. Tower, 20th floor, One Market Plaza, San Francisco, CA 94105 (US).		(81) Designated States: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SI, SK, TJ, TT, UA, UZ, VN, European patent (AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG), ARIPO patent (KE, MW, SD). Published <i>With international search report.</i>
(54) Title: METHODS FOR SCREENING ANTIESTROGEN COMPOUNDS		
(57) Abstract The present invention provides novel assay methods for identifying compounds that may have both estrogen agonist and antagonist properties. In particular, the assay use cells comprising promoters having an AP1 site linked to a reporter gene. Compounds capable of inducing or blocking expression of the reporter gene can thus be identified. The compounds may be further tested for the ability to modulate the standard estrogen response, as well.		

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METHODS FOR SCREENING ANTIESTROGEN COMPOUNDS

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BACKGROUND OF THE INVENTION

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Many breast tumors require estrogens for tumor growth. Thus, treatment with antiestrogen compounds can slow or prevent tumor spread. Many antiestrogens however, show both estrogen antagonistic and agonistic activity. The nonsteroidal antiestrogen tamoxifen, for example, which is established as the treatment of choice for the endocrine therapy of advanced breast cancer, shows both agonistic and antagonistic activity. Sutherland, S. & Jackson, M. *Cancer Treat. Revs.* 15: 183-194 (1987).

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The agonistic activity of tamoxifen and other antiestrogens may have profound effects upon patients. For example, agonistic activity may have beneficial effects, such as preventing osteoporosis and reducing serum cholesterol. Love, et al. *New Eng. J. Med.* 326: 852-856 (1992). Love, et al. *J. Natl. Cancer Inst.* 82: 1327-1332 (1990). Conversely, agonistic activity may also be harmful. Tamoxifen for example sometimes increases endometrial tumor incidence Iino, et al. *Cancer Treat. & Res.* 53: 228-237 (1991) or switches from inhibition to stimulation of estrogen dependent growth in breast tumor progression. Parker, M.G. (ed) *Cancer Surveys* 14: Growth Regulation by Nuclear Hormone Receptors. Cold Spring Harbor Laboratory Press (1992).

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It is desirable to identify pure antiestrogens as they are anticipated to provide more rapid, complete or longer-lasting tumor responses. Wakeling, A. E. *Breast Cancer Res. & Treat.* 25: 1-9 (1993). For example, ICI 164,384, thought to be a pure antiestrogen, blocked MCF-7 cell invasion activity of a re-constituted basement membrane while estradiol and 4'-hydroxytamoxifen stimulated this activity suggesting that early treatment of breast cancer with a pure antiestrogen might be particularly beneficial in limiting tumor spread. Braacke, et al., *Br. J. Cancer* 63: 867-872 (1991).

Conversely, while pure antiestrogens appear preferable for cancer treatments, mixed agonist-antagonist compounds may be preferable for preventative treatment. Such compounds should combine sufficient antagonist activity on estrogen stimulated breast tumor growth while maintaining simultaneous agonist activity on bone density and serum lipid levels.

Currently, antiestrogen compounds are screened with animal models such as the rat uterine test. These tests are cumbersome, slow, expensive and of uncertain application to humans because of differences between the human and rodent estrogen receptors.

Alternatively, investigators have used human breast cancer cell lines and assayed their growth as stimulated by estrogens or alternatively used the response of native or transfected reporter genes as assays of estrogen response. These cell lines utilize reporter genes that indicate activity of antiestrogen compounds that is mediated through the classical estrogen response element.

The prior art fails to provide methods for quickly and easily testing potential antiestrogen compounds for agnostic as well as antagonistic properties mediated through pathways other than the classical estrogen response pathway, that may affect, adversely or beneficially, their use in various therapeutic applications. This invention addresses these and other problems in the art.

SUMMARY OF THE INVENTION

The present invention provides methods for screening test compounds for the ability to activate or inhibit transcription through an indirect estrogen response or classical estrogen response. The indirect estrogen response is mediated by promoters comprising an AP1 site and the classical estrogen response is mediated by promoters comprising a classical estrogen response element. Preferred AP1 sites can be isolated from metalloprotease genes. Preferred classical estrogen response elements can be isolated from the *Xenopus vitellogenin A2* gene.

The methods typically use cells comprising an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene. The cells are then contacted with the test compound and the expression of the reporter gene is detected.

Cells used in the assay can be any cell which naturally expresses estrogen receptors or as the result of a transgene encoding the receptor. Preferred cells include MCF-7, ERC1, ERC2 or ERC3. The reporter genes used to detect an estrogen response include genes encoding beta-galactosidase and bacterial chloramphenicol acetyl transferase. The promoters used may be those which naturally comprise AP1 or estrogen receptor elements or the promoters may be genetically engineered to comprise those elements.

The assays may be used with cells comprising promoters with an AP1 site. Or alternatively a second set of cells with a promoter comprising a classical estrogen response element which regulates expression of a second reporter gene can be used. Alternatively, the two promoters and reporter genes can be in the same cells. In these assays the ability of test compound to induce or inhibit both the indirect and classical pathways can be determined.

When the methods are used to identify estrogen antagonists, the test compounds are contacted with the cells and a compound known to mediate an indirect estrogen response. The ability to inhibit the response is determined by detecting the expression of the reporter gene. Compounds known to mediate an indirect estrogen response include tamoxifen and estrogen at half maximal concentrations. The compounds can also be tested for the ability to induce or block the classical estrogen pathway, as well.

The invention further provides compounds identified by the claimed methods. Also claimed are cells which over-express an estrogen receptor and comprise a promoter comprising an AP1 site which regulates expression of a reporter gene. Exemplary cells include ERC1.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows estrogen stimulation mediated by an AP1 site. (A) A consensus AP1 site has ERE-like activity. Reporter genes, Δ coll73 and Δ coll60, were transfected into ERC1 cells (Kushner, et al., *Mol. Endocrinol.*, 4: 1465-1473 (1990) Δ coll73 consists of human collagenase promoter (-73 to +63) cloned upstream of the CAT gene. Angel, et al. *Mol. Cell. Biol.*, 7: 2256-2266 (1987). The position of the consensus AP1 site is indicated. Δ coll60 lacked the AP1 site (-73 to -60). ERE Δ coll60 contains an oligonucleotide corresponding to the frog vitellogenin A2 consensus ERE (Parker, M.G. (Ed), *Cancer Surveys 14 Growth Regulation by Nuclear Hormone Receptors*. Cold Spring Harbor Laboratory Press (1992)) immediately upstream of the collagenase sequences in Δ coll60. Typical results of CAT assays, normalized for transfection efficiency, following a single transfection are shown opposite. Each point is the mean value of triplicate assays, with standard errors. CAT activities from cells maintained in the absence of hormone are shown as white bars, those in the presence of a saturating concentration (100 nM) of estradiol as black bars. (B) Estrogen induction at the AP1 site requires AP1 proteins. Results of CAT assays prepared from F9 cells transfected with Δ coll73.

Figure 2 shows activity of AP1 sites in MCF-7 cells. Estrogen induction of AP1 dependent reporter genes Δ coll73 and Δ coll60 was described above. AP1 Δ TK-CAT consists of the collagenase AP1 site cloned upstream of a herpes simplex virus TK promoter (-32 to +45). TK-TATA lacks any known upstream transcription factor binding sites. CAT activities in the presence (black) and absence (white) of estrogen are shown opposite each reporter.

Figure 3 shows agonism by antiestrogens at the AP1 site. (A) CAT assays of ERC1 cells transfected with Δ coll73, Δ coll60 or ERE Δ coll60 and incubated with saturating concentrations (100nM) of estrogen (black), tamoxifen (shaded) and ICI (striped) or no hormone (white). A single experiment is shown, where the activities are the mean of three separate

points. (B) Dose dependence of antihormone agonism. ERC1, transfected with Δ coll73 were plated and exposed to a range of hormone concentrations. Each point is the mean of triplicate assays of CAT reactivity. (C) Antiestrogen activity in estrogen responsive cell lines. Results of representative CAT assays following transfection of Δ coll73 and Δ coll60 into MCF-7 cells and Δ coll73, Δ coll60 and ERE Δ coll60 into Ishikawa cells are shown. A single experiment is shown, where the activities are the mean of three separate points.

Figure 4 shows antiestrogen action in transient transfections. (A) Δ coll73 was introduced into CHO cells, with varying quantities of ER expression vector HEO (Holinka, C.F. et al., *J. Steroid Biochem.*, 25: 781-786 (1986) which is herein incorporated by reference), normalized to 10 μ g with the parent expression vector, SG5. CAT activities were determined in the presence of hormones, as in Figure 3A. (B) Requirement of ER domains for hormone action. The structure of four derivatives of the human ER cDNA is shown schematically. The DNA binding domain is indicated with the striped box, the ligand binding domain is marked E2. Transactivation functions TAF-1 and TAF-2 are marked. The right panel shows the effect of hormone upon Δ coll73 in the presence of each mutant. Transfections were carried out as described by Kushner, et al., 4: 1465-1473 (1990) which is herein incorporated by reference, in CHO cells, including 300ng of each ER expression vector.

DESCRIPTION OF THE PREFERRED EMBODIMENT

The present invention provides an efficient way to screen large numbers of test compounds for those which have desirable properties for either the treatment or the prevention of various cancers (e.g. breast cancer, ovarian cancer, endometrial cancer) and other diseases (e.g. endometriosis) mediated by estrogen. The invention allows for screening of test compounds for agonistic as well as antagonistic activity. In addition, this invention provides methods of screening for novel types of antiestrogen compounds that block only the indirect estrogen response and do not

block estrogen action at classical estrogen response elements.

In animals and in man the balance between stimulatory and inhibitory activities of antiestrogens such as tamoxifen varies widely depending on the organ, cell or specific protein measured as an indicator of estrogenic activity. This variety of effects is difficult to reconcile with the model of antagonism of estrogen receptor (ER) activity at classical estrogen receptor elements (ERs) as described in Beato, *M. Cell*, 56: 335-344 (1989) and Klein-Hitpass, et al., *Nucleic Acids Res.*, 16: 647-663 (1988) which are incorporated herein by reference.

The present invention relies, in part, on the discovery that ERs may activate transcription by interaction with another response element, the AP1 binding site, instead of binding to ERs. This AP1 mediated pathway, referred to here as the indirect estrogen response, may account for much of the agonistic properties of tamoxifen and other putative antiestrogens. A general description of the AP1 site is found in Angel & Kann, *Biochem. Biophys. Acta.*, 1072: 129-157 (1991) and Angel, et al., *Cell*, 49: 729-739 (1987), which are incorporated herein by reference.

In the methods of the invention, both the classical estrogen response elements and the indirect estrogen response may be used to provide a screening system that detects both estrogen antagonistic and agonistic activity. The methods typically comprise cultured cells that produce high levels of the human estrogen receptor. Preferred cells include MCF-7 cells or ERC1 cells described in Kushner et al., *Mol. Endocrinol.*, 4:1465-1473 (1990), which is incorporated herein by reference. ERC2 and ERC3 cells as described by Webb, et al. *Mol. Endocrinol.*, 6:157-167 (1993) which is herein incorporated by reference.

Other appropriate cells, including for example Ishikawa cells, and various breast cancer cells, which are known to one of skill in the art. The invention is not limited to practice in mammalian cells and may be practiced, for example in yeast and insect cells.

The cells may be modified to provide truncated or chimeric estrogen receptors as described in Berry, et al.,
115 *E.M.B.O. J.*, 9: 2811-2818 (1990) which is herein incorporated by reference. These modifications may result in increased estrogen affinity and increased sensitivity of the assay.

In addition, these cells are transfected with reporter genes in which a response element (either the AP1
120 site or ERE) regulates expression of a reporter gene. Typically, two different reporter genes are used. One gene reports transcription induced by the classical estrogen response system, while the other gene reports transcription induced by the indirect estrogen response. The two reporter
125 genes and response elements are typically placed in separate cells, but the methods can also be used with both constructs in the same cell.

The reporter gene for the classical estrogen response system contains an estrogen response element (ERE)
130 upstream of the target promoter and capable of regulating that promoter. In a preferred embodiment the ERE may be the consensus estrogen response element AGGTCACAGTGACCT from the *Xenopus vitellogenin A2* gene.

The particular ERE used in the cells is not a
135 critical aspect of the invention and the present invention is not limited to the use of this ERE. Other EREs known to one of skill in the art can also be used. For instance, other sources of naturally occurring EREs include the B2 gene, the chicken ovalbumin gene, and the PS2 gene. Alternatively, non-
140 naturally occurring EREs may be inserted into particular promoters. The consensus ERE from the *Xenopus vitellogenin A2* gene is widely used for this purpose, but other EREs may be used as well.

The reporter gene for the indirect estrogen response
145 pathway contains an AP1 site upstream of the target promoter and capable of regulating that promoter. The AP1 site is a sites that are bound by AP1 (the Jun and Fos proteins) or other members of that protein family. In a preferred embodiment, the consensus AP1 site is TGA(C/G)TCA.

150 One of skill would recognize that the particular AP1
site used is not a critical aspect of the invention. Any
sequence capable of being bound by AP1 or members of that
family and regulating a promoter is suitable. This would
include promoters which encompass a naturally occurring AP1
155 site. Typical promoters include, but are not restricted to
metalloprotease genes such as stromelysin, gelatinase,
matrilysin, and the human collagenase gene.

 Alternatively promoters may be constructed which
contain a non-naturally occurring AP1 or related binding site.
160 This facilitates the creation of reporter gene systems that
are not typically found under the control of AP1. In
addition, promoters may be constructed which contain multiple
copies of the AP1 site thereby increasing the sensitivity or
possibly modulating the response the reporter gene system.

165 The present invention is not limited to a particular
reporter gene. Any gene that expresses an easily assayable
product will provide a suitable indicator for the present
assay. Suitable reporter genes are well known to those of
skill in the art. They include, for example, bacterial
170 chloramphenicol acetyl transferase (CAT), beta-galactosidase,
or luciferase.

 To screen a number of compounds for antiestrogen
action, cells with high level expression of human estrogen
receptors and harboring either response element and reporter
175 genes are exposed to doses of estrogen which give half maximal
induction or less. In each case this will result in induction
of several to hundreds of fold depending on the levels of
estrogen receptor and the particular details of the reporter
construction. This will be reflected in increases of the
180 reporter gene product, such as the CAT gene product which may
be quantitated by enzymatic assay. The cells can be exposed
to estrogens either growing in separate wells of a multi-well
culture dish or for colorimetric assay in a semi-solid nutrient
matrix. Th compounds to be tested are added to the culture
185 dish wells or to small wells made in the semi-solid matrix and
the effect on the estrogen induction is assayed. An
antiestrogen compound will reduce or abolish the estrogen

induced increase in reporter gene activity. A hypothetical pure antiestrogen will block estrogen action with both types of reporter genes and will have no ability to induce the reporter genes in the absence of estrogen. A mixed estrogen antagonist-agonist, will show some ability to induce the reporter genes, especially the reporter genes linked to AP1 site.

In another embodiment, compounds which block the indirect pathway can be used to supplement tamoxifen or other antiestrogens in the treatment or prevention of breast cancer and other diseases mediated by estrogen. These compounds function to eliminate estrogenic agonistic activity of antiestrogens. Second they may have uses by themselves. In particular, it may be advantageous to block some estrogen mediated pathological effects at indirect estrogen response elements while leaving the direct pathway active. Compounds that block the indirect pathway are useful as components of combined oral contraceptives (COC) containing estrogens and progestins. A triple COC, containing estrogens, progestins, and a blocking compound would allow estrogen, either in the formulation or endogenous to act at the classical response elements, but would block action at the indirect response elements. Thus, a triple COC functions as current COCs to prevent pregnancy, but may also provide protective effects against breast cancer.

Typically, the reporter gene linked to the AP1 site is activated, not with estrogen, but with an excess (10 times the Kd) of tamoxifen, 4-hydroxy tamoxifen, or other antiestrogen. A library of compounds is searched for candidates that block or reduce reporter gene activation by the antiestrogen. The candidates are then tested with cells containing the reporter gene for the classical pathway to confirm that they do not interfere with estrogen activation at an ERE.

Compounds identified in the assays of the invention can be used in standard pharmaceutical compositions for the treatment of cancer, as components of oral contraceptives, or any other application in which the modulation of estrogen

activity is desired. The pharmaceutical compositions can be prepared and administered using methods well known in the art. The pharmaceutical compositions are generally intended for parenteral, topical, oral or local administration for prophylactic and/or therapeutic treatment. The pharmaceutical compositions can be administered in a variety of unit dosage forms depending upon the method of administration. For example, unit dosage forms suitable for oral administration include powder, tablets, pills, and capsules.

Suitable pharmaceutical formulations for use in the present invention are found in *Remington's Pharmaceutical Sciences*, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985), which is incorporated herein by reference. A variety of pharmaceutical compositions comprising compounds of the present invention and pharmaceutically effective carriers can be prepared.

The following example is offered by way of illustration, not by way of limitation.

EXAMPLE

The human collagenase gene, like other matrix metalloproteases, responds to AP1. Angel, et al., *Mol. Cell. Biol.*, 7: 2256-2266 (1987). To test whether an AP1 site could confer estrogen response the collagenase promoter was fused to the bacterial CAT gene (Δ coll73) and transfected into Chinese Hamster Ovary cells that overexpress ER (ERC1) Kushner, et al. *Mol. Endocrinol.* 4: 1465-1473 (1990).

Coll73 and Coll60, previously described by Angel, P. et al., *Mol. Cell. Biol.*, 7: 2256-2266 (1987) which is herein incorporated by reference, were modified by digestion with Eco0109 and Nde1 to remove an AP1 site in the backbone of pUC and were consequently designated Δ . ERE Δ coll60 was prepared by ligation of a consensus ERE, AGGTACAGTGACCT, into the HindIII site upstream of the Δ coll60 promoter. ERC transfections and electroporations were carried out as described in Kushner, et al., *Mol. Endocrinol.*, 4: 1465-1473 (1990) which is herein incorporated by reference. F9 cell transfections were by calcium phosphate coprecipitation. Cells were seeded at 30% confluence upon 1.5 cmn dishes and

transfected overnight with 5 mg reporter gene, 1 mg actin
265 B-HCG.

Included in some transfections, as indicated, were
100ng of AP1 expression vectors and 1 μ g of ER expression
vector HEO. Kumar, et al., *Cell*, 51: 941-951 (1987) which is
herein incorporated by reference. The human cfos expression
270 vector was BK28. Sassone-Corsi, et al., *Cell*, 54: 553-560
(1988) which is herein incorporated by reference. The human
c-jun expression vector was RSVc-Jun. Turner & Tjian,
Science, 243: 1689-1694 (1989) which is herein incorporated by
reference. The cells were glycerol shocked, re-fed and hormone
275 treated. All CAT assays were normalised to production of HCG
as described in Kushner, et al., *Mol. Endocrinol.* 4: 1465-1473
(1990). Data are expressed as counts per minute of tritiated
acetyl CoA converted per unit of HCG (CAT units).

Estradiol stimulated Δ coll73 ten fold (Fig. 1A),
280 whereas a similar reporter in which the AP1 site had been
removed (Δ coll60), gave reduced basal activity and no estrogen
response. Substitution of a classical ERE (Klein-Hitpass, et
al., *Nucl. Acids Res.* 16: 647-663 (1988)) for the AP1 site
(ERE Δ coll60), restored estrogen response, but not the elevated
285 basal activity.

In F9 cells, which lack endogenous AP1 activity
(Chiu, et al. *Cell*, 54: 541-551 (1988) which is herein
incorporated by reference) Δ coll73 failed to respond to
estrogen in the presence of transiently transfected ER (Fig.
290 1B). Estrogen activation could be restored by cotransfecting
expression vectors for AP1 proteins c-Jun and c-fos. Sassone-
Corsi, P. et al., *Cell*, 54: 553-560 (1988). Turner & Tjian,
Science 243: 1689-1694 (1989). In parallel, ERE Δ coll60 was
estrogen responsive, even in the absence of AP1, and Δ coll60
295 and remained unaffected by estrogen (data not shown).
Estrogen induction of the collagenase promoter therefore
required both the AP1 site and AP1 proteins.

To confirm that ER activates transcription through
AP1 sites under physiological conditions, we repeated the
300 study in MCF-7 (Fig. 2), a human breast tumor cell line in
which activity of the collagenase gene family is estrogen

responsive and related to tumor invasiveness. Thompson, et al., *Cancer Res.* 48: 6764-6768 (1988) which is herein incorporated by reference.

305 AP1 Δ TK-CAT consists of the collagenase AP1 site cloned upstream of a herpes simplex virus TK promoter (-32 to +45). TK-TATA lacks any known upstream transcription factor binding sites. AP1 Δ TK-CAT was constructed by ligation of an oligonucleotide spanning collagenase sequences -73 to -52 into
310 the HindIII site upstream of a TK-CAT derivative which had been modified to remove the PUC AP1 site. AP1 TK-TATA was constructed by ligation of the same oligonucleotide into the HindIII site of a CAT vector which contained TK sequences -32 to +45 and already lacked the PUC19 AP1 site Leitmann, D.C. et
315 al., *Mol. Cell Biol.*, 12: 1352-1356 (1992) which is herein incorporated by reference. MCF-7 transfections used the same protocol as ERC cells described in Kushner, et al., *Mol. Endocrinol.*, 4: 1465-1473 (1990).

 Estrogen stimulated Δ coll73 activity four fold, and
320 removal of the AP1 site abrogated estrogen responsiveness. The collagenase AP1 site also conferred estrogen response upon the herpes simplex virus TK promoter and the isolated TK TATA box, neither of which was estrogen responsive alone. Thus, the AP1 site was all that was required for estrogen response
325 on heterologous promoters. This type of estrogen induction may represent a far more common pathway than hitherto suspected. This pathway is referred to as the indirect estrogen response.

 The effects of antiestrogens on the indirect
330 estrogen response were examined next. All cells were transfected as described in Kushner, et al. *Mol. Endocrinol.*, 4: 1465-1473 (1990). Ishikawa cells, described by Holinka, et al., *J. Steroid Biochem.*, 25: 781-786 (1986) which is incorporated by reference, were induced with 10nM PMA and 10 μ M
335 forskolin prior to estrogen and antiestrogen induction.

 Tamoxifen inhibits estrogen action at classical EREs by interfering with the estrogen dependent transcription activation function in the ER ligand binding domain. Berry, et al., *E.M.B.O. J.*, 9: 2811-2818 (1990). It sometimes shows

340 partial agonism at classical EREs, which is attributed to
activity of a cell type specific constitutive transactivation
function in the amino terminus. *Id.* ICI inhibits a
dimerization function (Fawell, et al. *Proc. Natl. Acad. Sci.*
U.S.A. 87: 6883-6887 (1990)), which prevents high affinity
345 interaction with EREs in target promoters, and does not show
agonism in most assays. Wakeling, A. *J. Steroid Biochem.*
Molec. Biol., 37: 771-775 (1990).

Remarkably, both tamoxifen and ICI stimulated
 Δ coll73 expression five fold in ERC1 cells (Fig.3A). Neither
350 affected Δ coll60. In contrast, ERE Δ coll60 showed estrogen,
but not antiestrogens response indicating that antiestrogen
agonism is specific to the AP1 site. The dose response
(Fig.3B) of each agonist was consistent with its affinity for
the ER (Tora, et al., *E.M.B.O. J.*, 8: 1981-1986 (1989) (1nM
355 for estradiol, 10 nM for ICI and tamoxifen). We also examined
antiestrogen response in cells with endogenous ER. In MCF7,
tamoxifen weakly stimulated Δ coll73 expression and ICI showed
little effect (Fig.3C). A human endometrial cell line
(Holinka, et al., *J. Steroid Biochem.*, 25: 781-786 which is
360 herein incorporated by reference), estrogen, tamoxifen and ICI
were equally potent in eliciting an AP1 site-specific two fold
response but did not affect the classical ERE.

The effect of transiently expressed ER mutants
(Kumar, et al. *Cell*, 51: 941-951 (1987), Kumar & Chambon,
365 *Cell*, 55: 145-155 (1988) which is herein incorporated by
reference) upon Δ coll73 in CHO cells was examined to determine
which ER domains mediate an indirect response. Estrogen and
tamoxifen activated the collagenase promoter (Fig.4A),
although both were less effective with high amounts of
370 transiently transfected ER. ICI induction became more
significant at high ER contents. This is consistent with
observations that ICI reduces ER levels by reducing the half
life of ER protein. Dauvois, et al., *Proc. Natl. Acad. Sci.*
U.S.A., 89: 4037-44041 (1992).

375 The ER contains two transcriptional activation
functions (Fig.4B), amino terminal constitutive (TAF-1), and
carboxyl terminal estrogen dependent (TAF-2). Isolated TAF-1

(HE15) appears to give hormone independent activation of transcription and deletion of TAF-1 (HE19) reduced estrogen response and abolished antiestrogen activity. Antiestrogen activity upon Δcoll73 in CHO cells therefore reflects TAF-1 activity. This agrees with suggestions that tamoxifen cannot activate TAF-2 (Berry, et al. (1990)) and further implies that ICI cannot activate this function. ICI agonism may also imply that monomeric ER functions in this complex. Fawell, et al. (1990). Deletion of the DNA binding domain (HE11) did not reduce hormone induction suggesting that the response is independent of DNA binding and rather mediated indirectly through AP1 proteins.

In summary, indirect estrogen response is widely active, and antiestrogens are agonists of this pathway. It is possible that any of the well described agonist effects of tamoxifen reflects indirect estrogen response. Antiestrogens would have estrogenic activity on critical AP1 regulated target genes, hence growth and differentiated response, in cells in which ER and AP1 proteins could interact. Changes in AP1 during tumor progression could be particularly significant, and should be considered in models of antiestrogen resistance in breast cancer. Parker, et al. *Cancer Surveys*, 14, Growth Regulation by Nuclear Hormone Receptors. Cold Spring Harbor Laboratory Press (1992).

Although the invention is described in some detail in the above description and examples for the purposes of clarity and understanding, it will be apparent that certain changes and modifications can be made within the scope of the attached claims.

WHAT IS CLAIMED IS:

1. A method for screening a test compound for the ability to activate transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound; and

c) detecting the expression of the reporter gene.

2. A method of claim 1, wherein the cell is a MCF-7 cell.

3. A method of claim 1, wherein the cell over-expresses the estrogen receptor.

4. A method of claim 3, wherein the cell is an ERC1 cell.

5. A method of claim 1 wherein the reporter gene encodes beta-galactosidase.

6. A method of claim 1 wherein the reporter gene encodes bacterial chloramphenicol acetyl transferase.

7. The method of claim 1, wherein the promoter is genetically engineered to comprise an AP1 site.

8. A method of claim 1, further comprising the steps of:

a) providing a second cell comprising an estrogen receptor and a promoter comprising a standard estrogen response element which regulates expression of a second reporter gene;

b) contacting the second cell with the test compound; and

c) detecting the expression of the second reporter gene.

9. A method of claim 8, wherein the response element is from the *Xenopus vitellogenin A2* gene.

10. A method of claim 1, wherein the cell further comprises a promoter comprising a standard estrogen response element which regulates expression of second reporter gene.

11. A method of claim 10 wherein the response element is from the *Xenopus vitellogenin A2* gene.

12. An estrogen agonist identified by the method of claim 1.

13. A method for screening a test compound for the ability to inhibit transcription through an indirect estrogen response, the method comprising:

a) providing a cell comprising an estrogen receptor and a promoter comprising an AP1 site which regulates expression of a reporter gene;

b) contacting the cell with the test compound and a compound known to mediate an indirect estrogen response;

c) detecting the expression of the reporter gene.

14. The method of claim 13, wherein the compound known to mediate an indirect estrogen response is tamoxifen.

15. The method of claim 13, wherein the compound known to mediate an indirect estrogen response is estrogen at half maximal concentration.

16. A method of claim 13, wherein the cell is a MCF-7 cell.

17. A method of claim 13, wherein the cell over-expresses the estrogen receptor.

18. A method of claim 17, wherein the cell is an ERC1 cell.

19. The method of claim 13, wherein the promoter is genetically engineered to comprise an AP1 site.

20. A method of claim 13, further comprising the steps of:

a) providing a second cell comprising an estrogen receptor and a promoter comprising a standard estrogen response element which regulates expression of a second reporter gene;

b) contacting the second cell with the test compound; and

c) detecting the expression of the second reporter gene.

21. The method of claim 20, further comprising the step of contacting the second cell with a compound known to mediate a standard estrogen response.

22. A compound identified by the method of claim 13.

23. A composition comprising a cell which over-expresses an estrogen receptor and comprises a promoter comprising an AP1 site which regulates expression of a reporter gene.

24. The composition of claim 23, wherein the cell is ERC1.

1/4

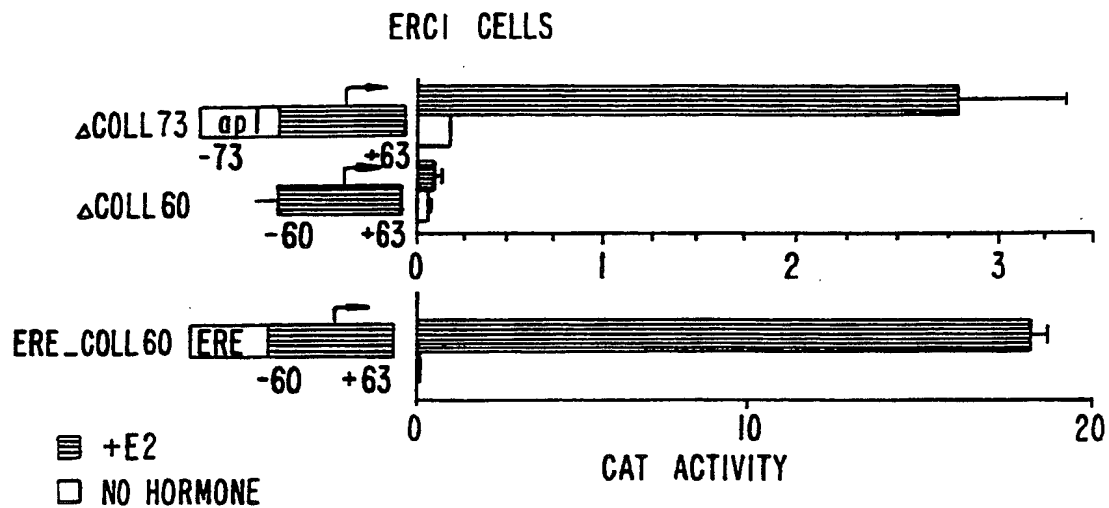


FIG. 1A.

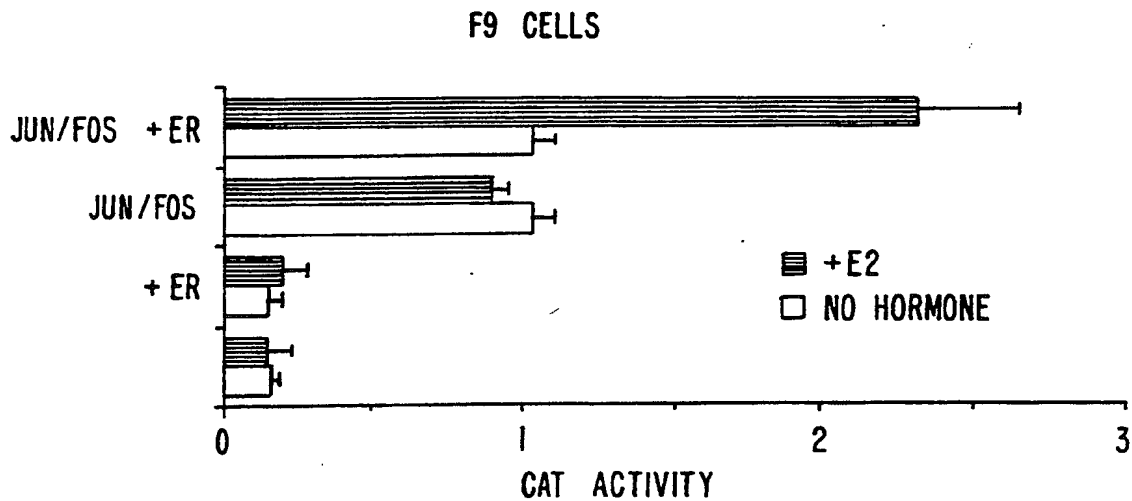


FIG. 1B.

2/4

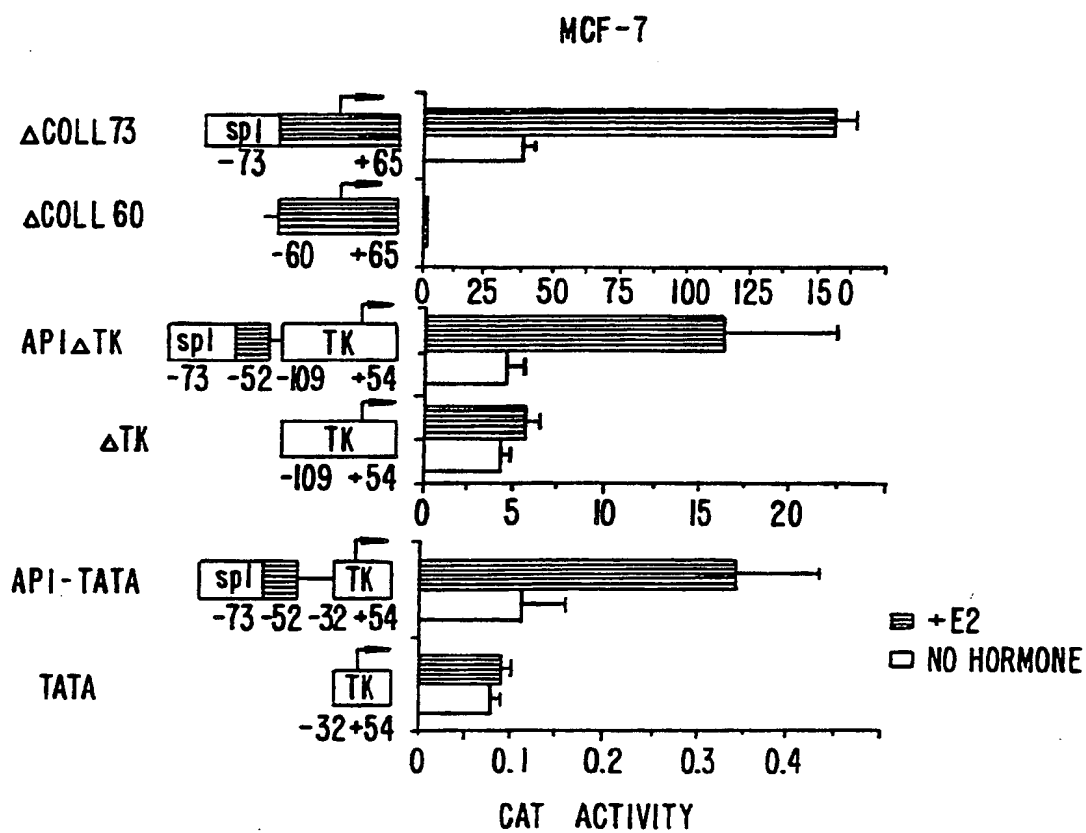
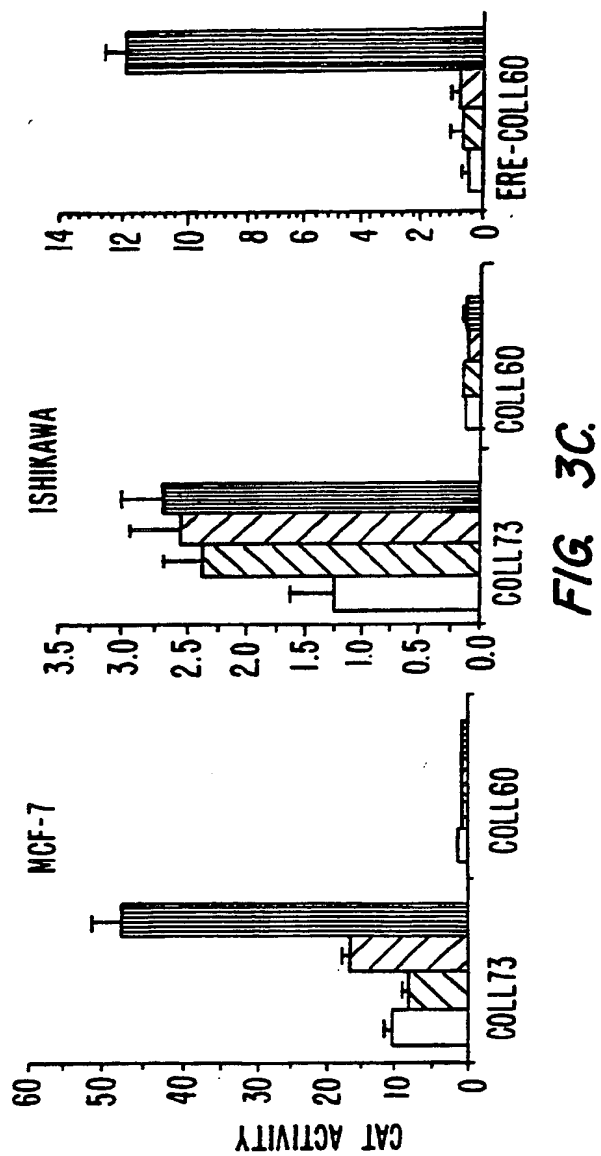
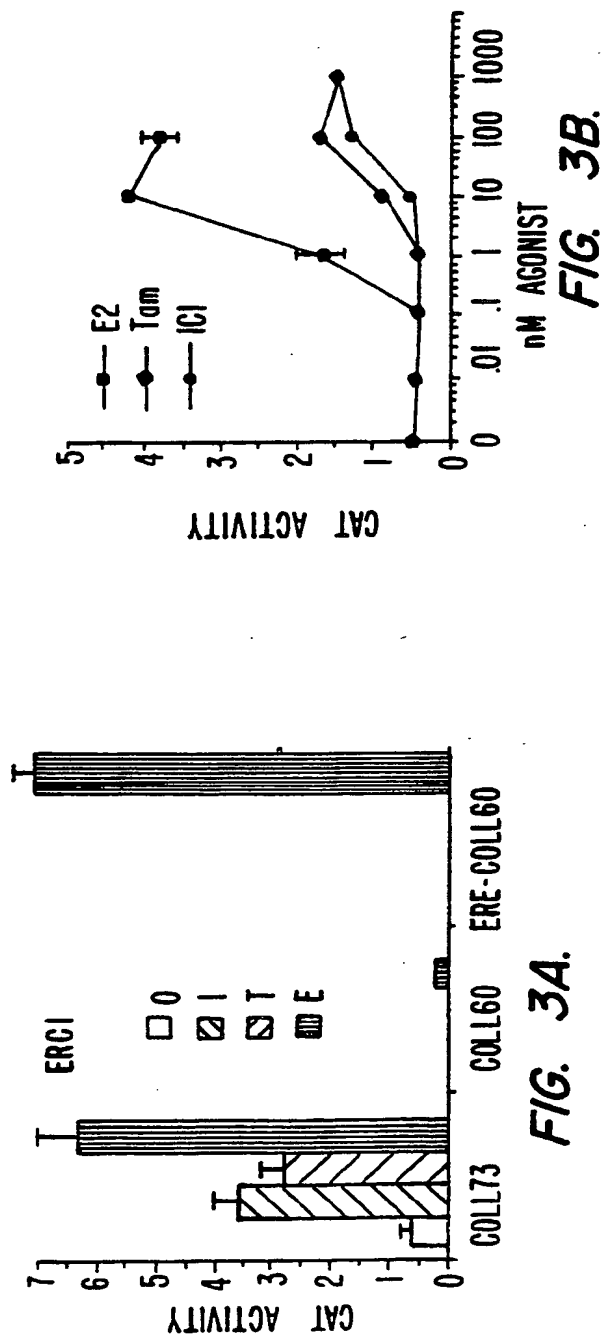


FIG. 2.

3/4



4/4

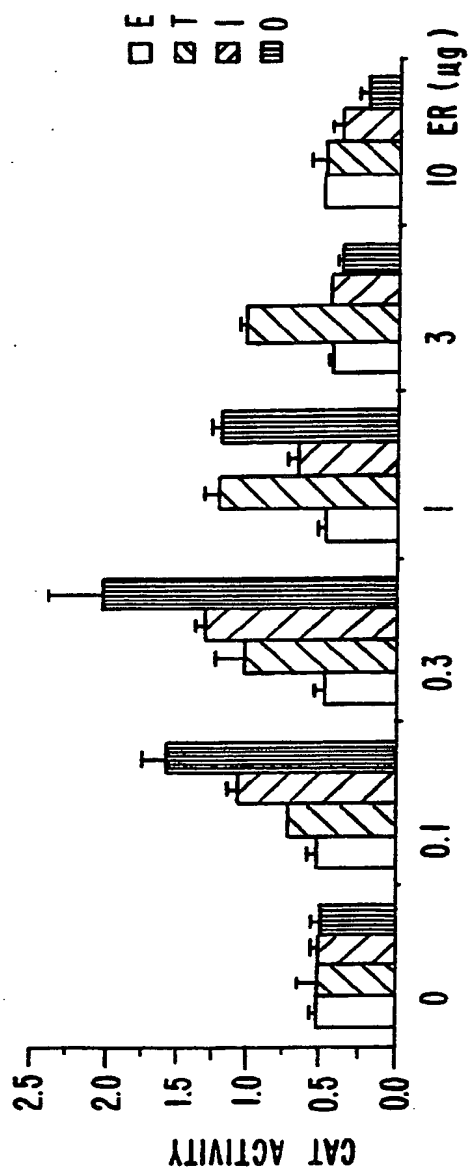


FIG. 4A.

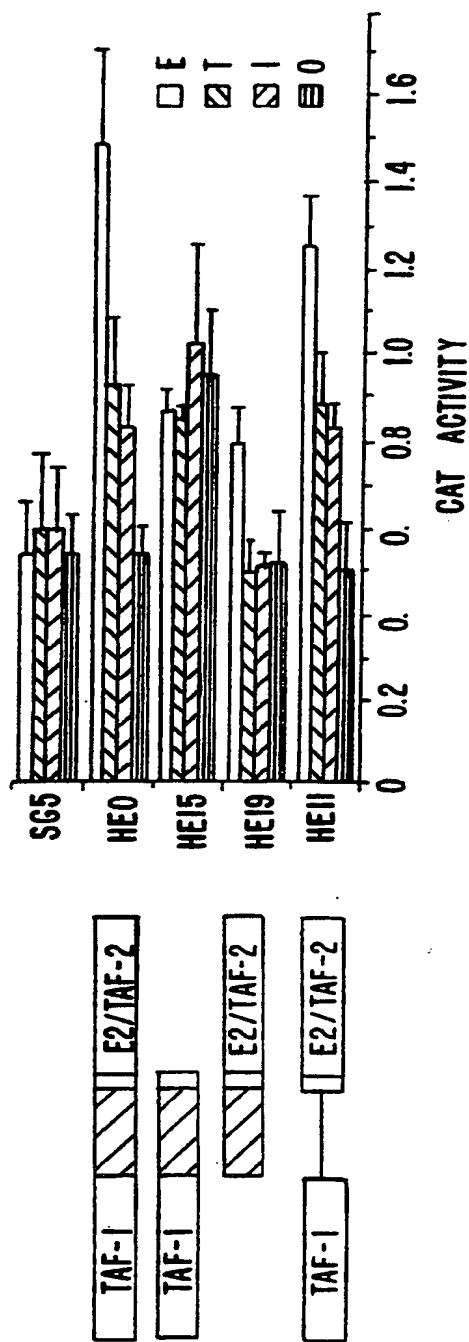


FIG. 4B.

INTERNATIONAL SEARCH REPORT

 International application No.
 PCT/US94/09898

A. CLASSIFICATION OF SUBJECT MATTER

 IPC(6) : C12Q 1/68, 1/00; C12P 21/06; C12N 15/00, 1/20; G01N 33/566; C07K 3/00.
 US CL : 435/6, 7.2, 69.1, 172.3, 252.3; 436/501; 530/350
 According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/6, 7.2, 69.1, 172.3, 252.3; 436/501; 530/350

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

 Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
 APS, DIALOG - BIOTECH FILES

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim N .
X --- Y	Journal of Biological Chemistry, Volume 268, No. 19, issued 05 July 1993, A. Philips et al, "Estradiol Increases and Anti-estrogens Antagonize the Growth Factor-induced Activator Protein-1 Activity in MCF7 Breast Cancer Cells without Affecting c-fos and c-jun Synthesis", pages 14103-14108, see entire document.	1-3, 6-11, 13-17, 19-21, 23 ----- 4, 5, 12, 18, 22, 24
Y	BioTechniques, Volume 9, No. 4, issued 1990, M. Pons et al, "A New Cellular Model of Response to Estrogens: A Bioluminescent Test to Characterize (Anti)Estrogen Molecules", pages 450-459, see entire document.	1-24

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be of particular relevance	*X*	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y*	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G*	document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means		
P document published prior to the international filing date but later than the priority date claimed		

 Date of the actual completion of the international search
 01 NOVEMBER 1994

 Date of mailing of the international search report
 DEC 02 1994

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US94/09898

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Cell, Volume 63, issued 21 December 1991, M. -P. Gaub et al, "Activation of the Ovalbumin Gene by the Estrogen Receptor Involves the Fos-Jun Complex", pages 1267-1276, see entire document.	1-24
Y	Molecular Endocrinology, Volume 4, No. 10, issued 1990, P. J. Kushner et al, "Construction of Cell Lines that Express High Levels of the Human Estrogen Receptor and Are Killed by Estrogens", pages 1465-1473, see entire document.	4, 5, 12, 18, 22, 24